PALL Life Sciences

Application Note

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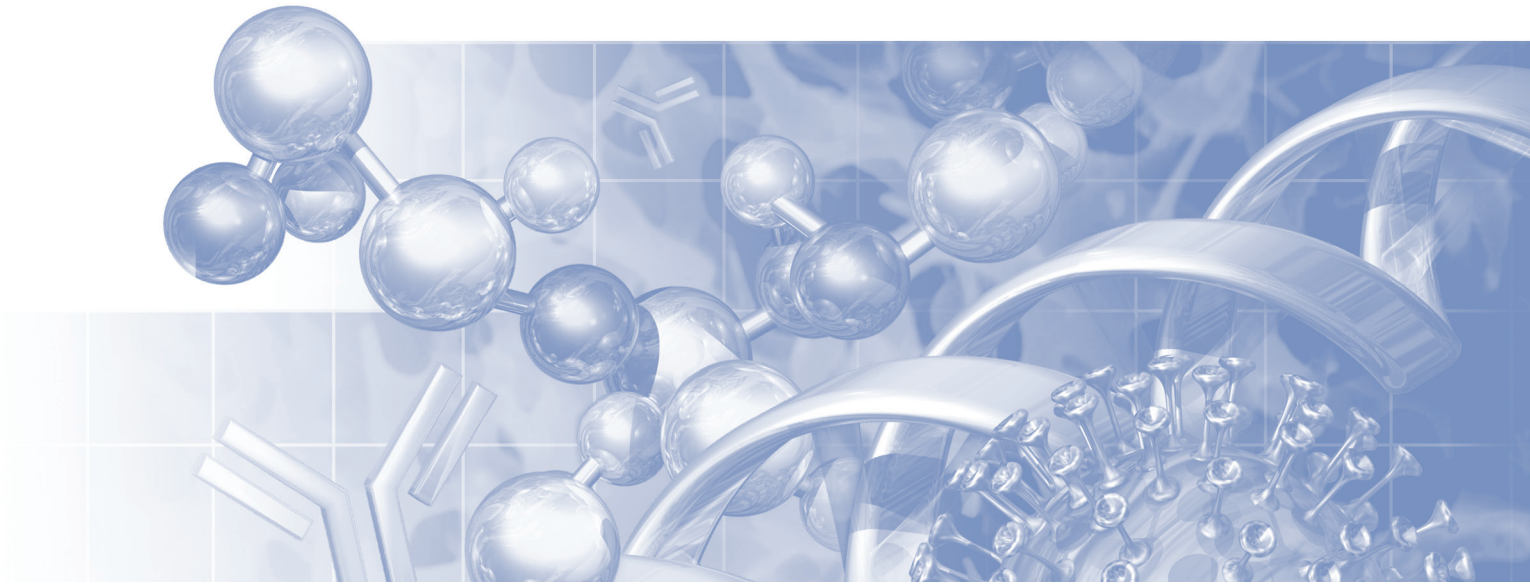
Cadence™ Virus Inactivation System

Automated semi-continuous low pH virus-inactivation on a single-use mixing platform



*Filtration. Separation. Solution.*SM

Abstract

This document describes the capability of the Cadence virus inactivation (VI) system. The system is designed to perform low pH virus inactivation of monoclonal antibodies (mAbs) and recombinant proteins in a semi-continuous manner, simplifying virus inactivation of biologics in a continuous processing stream. By automating the VI process, advantages of continuous processing can be realized. Additionally, automation of the process reduces the risk of operator error, contamination and process deviations.

Introduction

Demonstrating virus safety is a regulatory requirement for biologics expressed in human or animal cell lines due to the increased propensity for endogenous and adventitious viral contamination. Three complementary approaches are used to control the risk of viral contamination of biotechnology-derived products[1]:

1. Selecting and testing cell lines and screening of raw materials, including media components.

2. Assessing the capacity of the production processes to clear infectious viruses.

3. Testing the product at appropriate steps of production for absence of contaminating infectious viruses.

As it is impossible to know and test the complete range of viruses that might infect the cells, studies are performed with relevant model viruses to assess the viral clearance capability of the downstream purification process. The goal being to illustrate that the manufacturing process can clear the relevant viruses by inactivation or removal and, by inference, is sufficiently robust for clearing a broad range of viruses[2].

Typically, virus clearance is achieved by two (or more) orthogonal steps, virus filtration, and the step we will focus on here, low pH virus inactivation. ASTM E2888 – 12[3] states that this latter process should assure 5 $\log_{10}$ inactivation of non-defective C-type retroviruses when performed under the following parameters: Hold temperature of ≥ 15 °C, hold time of ≥ 30 minutes, and pH of ≤ 3.6 throughout the course of the hold.

Low pH virus inactivation, that typically follows Protein A capture chromatography step, is well understood and well-characterized for a batch process where the pH adjustment steps are performed either manually using off-line pH measurement or using automated/semi-automated equipment with in-line pH measurement and acid/base dosing.

For a continuous Protein A capture chromatography process, that utilizes a multicolumn chromatography system such as the Cadence BioSMB process 350 or 80 systems, multiple Protein A elutions are expected each hour and processes might take place over multiple shifts. In order to maintain the cadence of a continuous process beyond the Protein A capture chromatography step, it is advisable to investigate approaches that enable a semi-continuous or a continuous virus inactivation method. Two basic approaches are viable for virus inactivation a) a continuous plug flow reactor with in-line pH reduction, hold and neutralization and b) a multi-vessel continuously stirred-tank reactor (CSTR) with *in-situ* pH reduction, hold and neutralization. While the CSTR approach generates a pause in the continuity in flow at the start of the process, there are numerous advantages of this approach that include:

i) Identical to the well-known and well understood low pH inactivation method commonly employed by the industry and accepted by the regulatory authorities.

ii) Collection of elution pool enables homogenization of the protein and therefore eliminates any impact of protein concentration gradient on virus inactivation and also simplifies virus clearance validation studies.

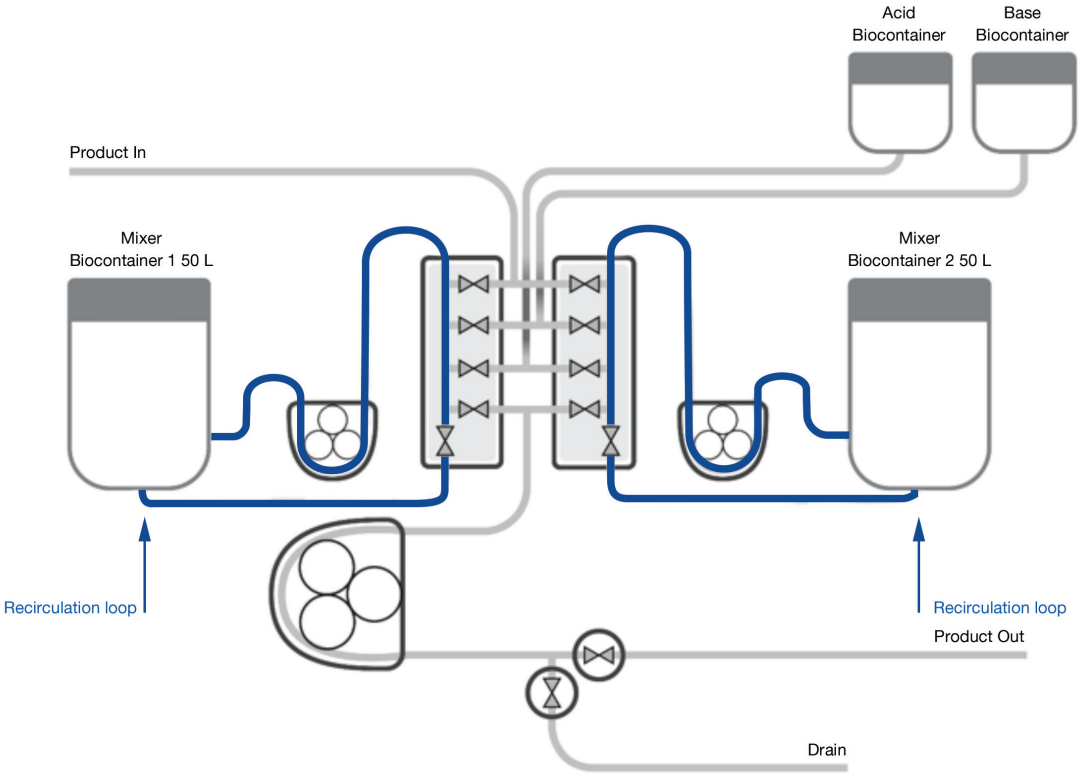
iii) Approach is transferable to other inactivation methods using solvent/detergent, etc. where the volume of the inactivation agent(s) are well characterized.

iv) CSTR serves as a suitable hold vessel for homogenization of solution conditions in order to prepare it for the subsequent purification steps.

Here we describe an automated, semi-continuous system for performing low pH virus inactivation using the CSTR approach. The system has two mixers that are used alternately and asynchronously. A pool of elutions is collected in one of the mixers. The complete VI process, acidification, hold and pH increase, takes place in that mixer. While the mixer undertakes the titration and low pH hold, the other mixer is receiving new elutions. When the inactivation is complete the elution pool is transferred out, and the mixer is ready to collect more elutions, allowing the other mixer to perform the inactivation cycle. Figure 1 shows the schematic of the Cadence virus inactivation system. A failure mode and effects analysis (FMEA) risk assessment was performed to identify critical components and features of the system to be tested. In this document we will present the applications data that characterize individual components of the system; the pH probes, the mixer and the pump used to empty the mixer tank. Then we will show data which demonstrates that the system is functionally free of hold-ups which can result in contamination of inactivated product. Finally, we will show data for the robustness of the automated operation of the system for 24 hours.

Figure 1

Schematic of the Cadence virus inactivation system.



Characterization of Key Components

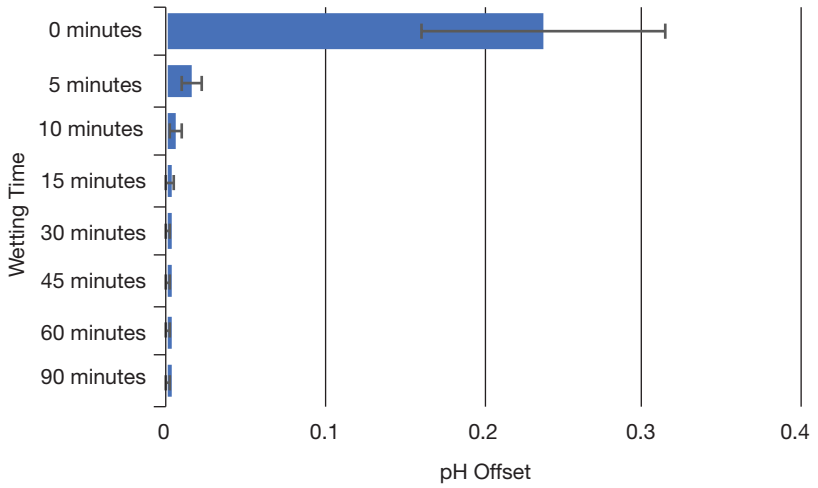
pH probes

The pH probes are a key element of the virus inactivation system. It is necessary for the probes to remain precise and accurate through the course of a continuous process as pH is a critical process parameter for achieving sufficient virus inactivation[4]. Additionally, there is a challenge in calibrating the probes in a single-use system: It is inconvenient to pause operations to use a probe calibration solution, in the flow path of the product, when that product is part of a continuous process.

The Cadence virus inactivation system includes Mettler Toledo◆ probes (LGRMX225PH). These probes are calibrated and then autoclaved before sterile installation using an aseptic bellows design. Data shows that this process results in a period of ‘wetting in’ time for the probe to read accurately after being autoclaved which manifests as a decreasing pH offset between the pH of the solution and the value the probe reads as the probe becomes wetted (Figure 2).

Figure 2

Graph showing the 'wetting in' time for the pH probes after autoclaving against the deviation from actual pH (pH offset). Triplicate measurements were made and the error bars represent the standard deviation.



In testing the pH probe proved to be reading within tolerance after a wetting period of approximately 10 minutes. However, in some circumstances it may be necessary to adjust the pH probe to calibrate against drift in the response. To address this we designed the system to be capable of making a single point offset of the pH probe to improve the accuracy of the measurement. Here we recommend making the offset whilst the mixer is in the filling phase, ideally between elutions before the first titrations take place.

The system enables a sample to be taken via a sampling port and the pH transmitter software allows the pH offset to be taken against an earlier time point. This allows the pH of the sample to be measured offline and then the offset made at the time the sample was taken, so that if the pH in the tank has subsequently changed the offset is still relevant. The offset measurement should be taken at least 10 minutes after the probe is fully covered in liquid, to ensure that the probe reading is stable.

The reliability of the pH probe measurement is a key requirement for a continuous VI system. Here we tested the operation of the probes over time in a way that simulated the actual VI process. An automated test rig was designed to assess the probes, mimicking the VI process by transferring the probes between two test solutions:

1. Human IgG 10 g/L in 20 mM acetate pH 3.5
2. Human IgG 10 g/L in 20 mM Tris pH 8.0

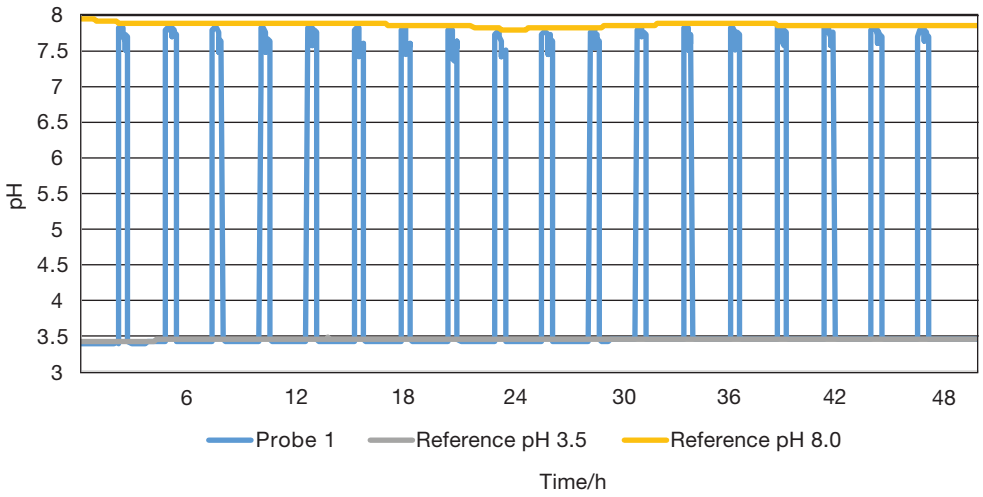
These simulate a typical product at the VI hold step (pH 3.5) and mimic the pH after neutralization (pH 8) respectively. The test rig was designed to move the probes from one solution to the other with the following sequence to mimic an actual VI process where the pH is probes are serially wetted and dewetted as the biocontainers empty and fill.

1. Solution 1 (pH 3.5) for two hours
2. Solution 2 (pH 8) for 15 minutes
3. Exposed to air for 15 minutes (mimicking the time the probe would be uncovered in the actual system as the tank is emptied and then the next elution arrives)
4. Back to step 1

Reference probes were used to monitor the pH of the two solutions over time. This experiment was performed for a total duration of 48 hours. The data is shown in Figure 3.

Figure 3

Shows the performance of the pH probe vs. the reference probe of a 48 hours in an automated process designed to mimic the low pH virus inactivation step.



It is apparent from the data that the probes exhibited minimal drift through the course of the experiment. We can look at measurements made at the beginning of the experiment, at the middle of the experiment (at 24 hours) and at the end of the experiment (after 48 hours) and see that the pH probes accuracy remains well within 0.1 pH units, Table 1.

Table 1

Shows the pH as measured by the test probe vs. a reference probe for the beginning, middle and final low pH hold step in a 48 hour test.

	Start			24 h			48 h		
pH Probe	Test Probe	Reference Probe	Difference	Test Probe	Reference Probe	Difference	Test Probe	Reference Probe	Difference
1	3.569	3.5868	0.0178	3.5800	3.5821	0.0021	3.5949	3.5925	0.0024
2	3.3728	3.3874	0.0146	3.3874	3.4000	0.0126	3.4010	3.3999	0.0011
3	3.4104	3.4187	0.0083	3.4291	3.4260	0.0031	3.4382	3.4381	0.0001
4	3.5063	3.5182	0.0119	3.5253	3.5332	0.0079	3.5281	3.5270	0.0011
5	3.4332	3.4314	0.0018	3.4461	3.4430	0.0031	3.4489	3.4438	0.0051
		Mean	0.0075		Mean	0.0058		Mean	0.0020
		Standard Dev.	0.0061		Standard Dev.	0.0044		Standard Dev.	0.0019
		Total Error	0.0197		Total Error	0.0146		Total Error	0.0058

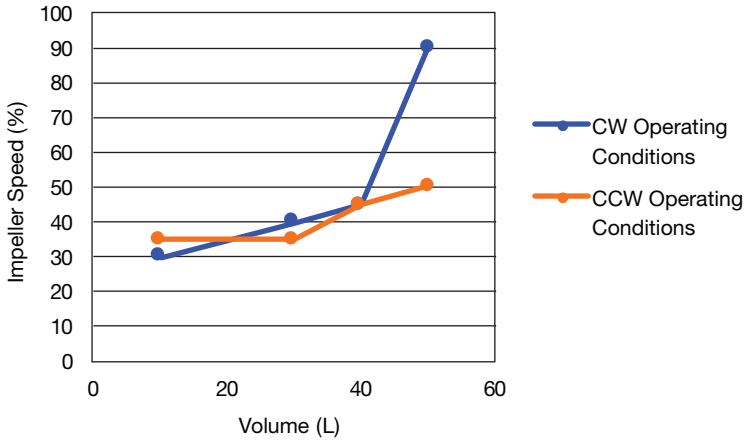
Characterization of the Allegro™ 50 L Mixer

The Allegro 50 L (P/N VICAD-LGR-50) mixer is designed to provide exceptional mixing performance for a wide range of applications from upstream through downstream process to formulation and filling. This particular model of mixer is dedicated to the VI system allowing safety interlocks with the main control system and easy sampling. Due to its efficient mixing and high turn-down ratio, this mixer is especially suited to small volume formulation applications where fast, reproducible results are required.

Here we wanted to test how quickly we could achieve mixing without causing foaming. Tests were performed with 10 g/L IgG in phosphate buffered saline (PBS) at a series of different volumes in the mixer, namely 10, 30, 40 and 50 L with mixing investigated in both the clockwise (CW) and counter-clockwise (CCW) directions. In these studies the mixer was held at a certain speed for a period of 5 minutes to visually assess foaming. If no foaming was observed the speed of the mixer was increased and foaming re-assessed. The outcome of this study is shown in Figure 4.

Figure 4

Data showing the maximum mixing speed that can be used before mixer induces foaming in an IgG solution. CW: clockwise direction; CCW: counter-clockwise direction.



After the maximum usable mixer speed, at which foaming does not occur, was determined the mixing efficiency was assessed for the clockwise and counter clockwise operation at the maximum working volume (50 L) and a lower working volume (10 L). The mixing time was determined using a conductivity probe and measuring the time it took for the conductivity to become stable after an addition of NaCl via the top port of the mixer. The data is shown in Table 2.

Table 2

Mixing times with clockwise (CW) and counter-clockwise (CCW) mixer direction.

Volume (L)	Impeller Speed	Direction	Time (sec)
10	35	CCW	14
10	30	CW	8
50	50	CCW	22
50	90	CW	17

Mixing times are longer with CCW operation, but mixing is still achieved in under 30 seconds without the risk of foaming. CCW was chosen as the default mixing direction as the maximum mixing speed without foaming is less volume dependent than CW operation.

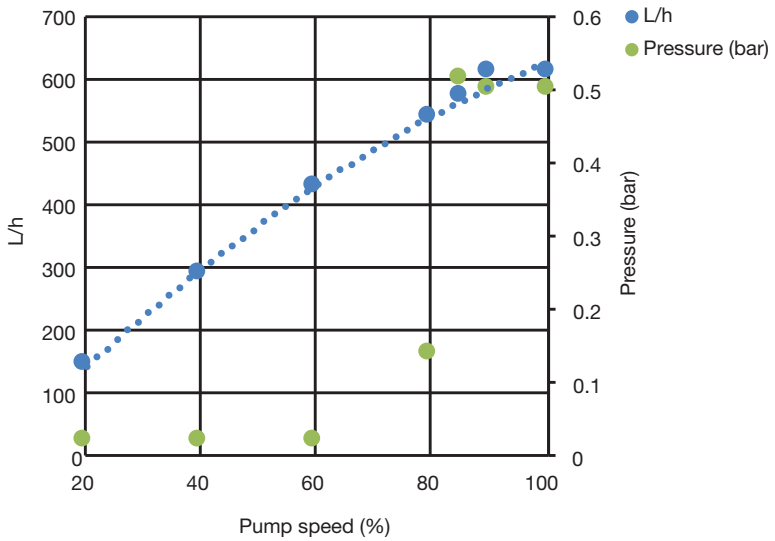
Transfer Pump Flow Rate Limit

The system has two mixers that are used alternately and synchronously. The total VI process has to take place before the alternate mixer tank becomes filled with elutions. Therefore, the flow rate of incoming elutions that can be processed by the system in a given time is defined by the maximum mixer volume minus the titration volume divided by the time it takes to adjust the pH down, perform low pH hold, adjust the pH back up and empty the mixer.

For the system to be at its most efficient it is necessary to empty the mixers, after low pH processing, as quickly as possible. Longer emptying times reduce the effective volume that can be processed by the system. The pump was tested at increasing speed to maximize mixer emptying, Figure 5, as the pump speed increases to 80% the mixers can be emptied at >500 L/h. This means a mixer at the maximum working volume can be emptied in < 6 minutes without significant pressure increase. Use of the pump above 80% is not recommended as the liquid flow appears to enter a turbulent state. A 6 minute empty time is consistent with the overall planned capacity of the system.

Figure 5

Effect of pump speed on the flow rate that mixers can be emptied.

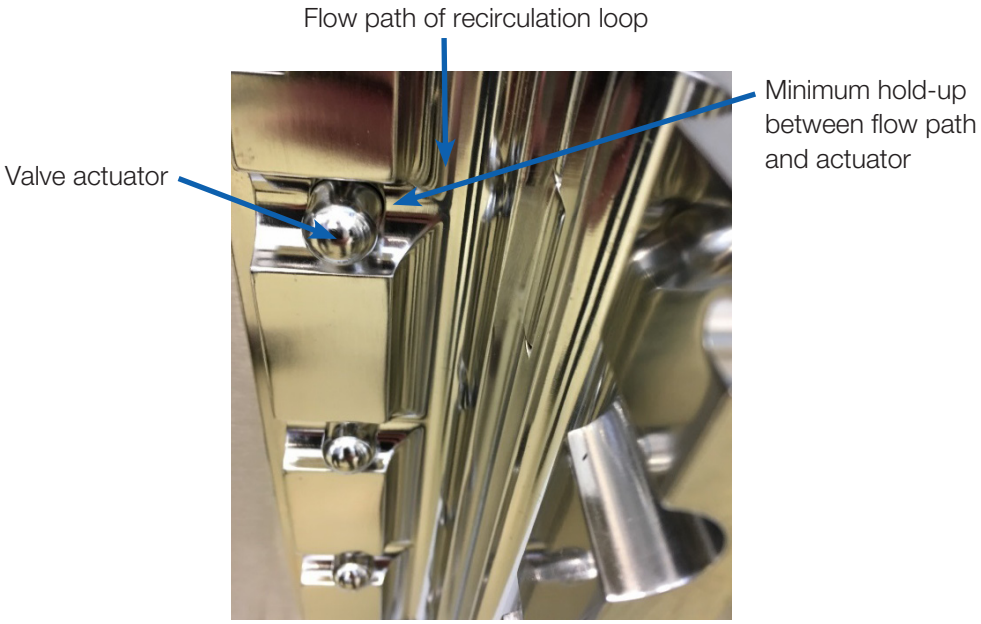


Hold-Up Testing

The Cadence VI system was set up with two 50 L Allegro mixers that are used alternately and asynchronously. The complete VI process, acidification, hold and pH increase, takes place in a single tank. While one mixer is receiving new elutions, the other mixer undertakes the titration and low pH hold, Figure 1. This poses a potential risk that liquid that has not been treated by acidification may come into contact with acidified product, resulting in contamination with active virus, a common concern with top down entry. To minimize this risk, the system has been designed with low point product entry to minimize splashing. Acid and base are also added below the liquid level and to accommodate for this a recirculation loop is used to prevent mixing with acid/base whilst the incubation step is ongoing. Additionally, to minimize hold-ups Aquasyn◆ valve blocks are employed which feature a very low hold-up design (see below).

Figure 6

Image showing valve actuator without the single-use manifold. The image highlights swept flow path within the Cadence VI valve block.



In order to validate these design choices, as well as to demonstrate that the Cadence VI system is free of hold-ups, a series of tests were performed. Riboflavin was used as a visual test for hold-ups and the bacterium *Brevundimonas diminuta* was used to thoroughly scrutinize the system for dead-legs. Finally, experiments were performed with the bacteriophage Phi6, to mimic a mammalian virus.

Riboflavin Test for Hold-Ups

Riboflavin is a standard reagent that is used to assess a system for hold-ups[5]. Riboflavin has ultraviolet (UV) absorbance maxima at 222, 263 and 373 nm. The 373 nm absorbance is close to commonly used 'black-lights' which emit at 365 nm. When illuminated at 365 nm riboflavin fluoresces bright yellow and its presence after washing and cleaning is indicative of incomplete cleaning or hold-ups. Riboflavin is chosen due to the very low limit of detection.

To check for hold-ups a 0.02 g/L riboflavin solution was prepared in 1X PBS. This was the highest concentration that was soluble.

The mixers were filled with riboflavin solution, drained and then filled with water, mixed for 10 seconds and then drained again. Two different operators installed four different mixer biocontainers for a total of six different tests. The presence of riboflavin was visually monitored through the course of the experiments using illumination at 365 nm.

No riboflavin was found above the original liquid level and in the upper portions of the biocontainer, giving high confidence that the no splash back and low foaming design of the recirculation loop and entry points have eliminated the risk of carry-over of active virus.

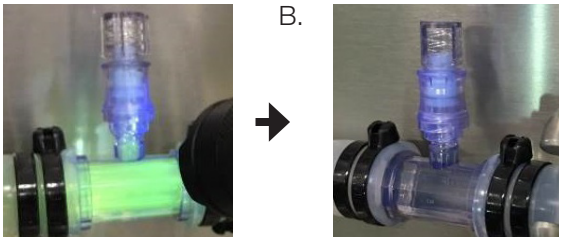
An additional observation was made, in two of the tests after the riboflavin had been drained and before the water rinse, a small amount of riboflavin could be observed in folds in the biocontainer. This indicates, as may be expected with a single-use biocontainer, that the draining is not 100%. In terms of the VI this means that a small amount of inactive elution will remain in the biocontainer and will be subject to a second treatment. The volume remaining in the biocontainer and tubing manifold after draining is estimated to be 100 mL. After refilling and subsequent emptying of the biocontainer no riboflavin could be detected.

Additionally potential hold-ups in the rest of the manifold were also investigated with riboflavin. Here we show photos of the sampling port in the recirculation loop before and after recirculation. No hold-up of the riboflavin could be detected.

Figure 7

Sampling port does not contain a hold-up. A) Image of sampling port with riboflavin. B) Image of sampling port after buffer is pumped through the recirculation loop.

A. B.



Bacterial Challenge Testing for System Hold-Ups

To further address concerns over potential hold-ups in the system, the bacterium *Brevundimonas diminuta* (ATCC 19146) was selected as a surrogate for mammalian virus. The advantages of bacterial testing include increased method sensitivity (ability to detect 1 colony forming unit (CFU) in any given volume) and the ability to sample larger volumes by collecting bacteria via filtration before assaying.

Bacterial testing was performed over two continuous low pH cycles in a single mixer. After these two cycles were completed, sterile Tryptic Soy Broth (TSB) was added to the mixer via the top port of the mixer biocontainer. This was aseptically collected, and 2 L (1/3 of the volume) was analyzed by membrane filtration to assess if there is the potential for carry-over of any viable organisms.

This test required a lower pH (pH 2.5) than is typically used for VI as *B. diminuta* is not killed at pH 3. Using this low pH is consistent with the objective of the experiment to assess if dead-legs could reduce the effectiveness of the low pH treatment. By ensuring 100% kill of the bacteria that are in direct contact with the low pH conditions, and sampling a large volume of growth media recovered at the end of the study (post-acidification), this allows a thorough investigation into the possibility of bacteria being held up in the system at a very low level of detection of 1 CFU/2 L.

Test Method

1. Fill mixer with 10 L 50 mM citrate phosphate buffer spiked with *B. diminuta* to a target concentration of 5 x 10^6 CFU/mL. Retain a sample to determine initial starting concentration ('nput').
2. Perform VI at pH 2.5 for 60 minutes.
3. Neutralize, add 1M Tris base to pH 4.5.
4. Pump out liquid (through transfer pump pathway and standard VI method), and aseptically collect into a suitable sterile container (e.g. single-use biocontainer or equivalent).
5. Repeat steps 1-3 to complete two VI cycles in one mixer.
6. Fill mixer 1 with 6 L of sterile TSB using top port. Pump the solution around the recirculation loop.
7. Drain using the transfer pump and aseptically collect into a suitable container ('Post Cleanliness').
9. Test for *B. diminuta*: Pass 2 L of the collected TSB through a sterile (0.2 µm rated) recovery membrane using vacuum filtration. Once the volume has been passed, aseptically transfer the recovery membrane to a Trypticase Soy Agar (TSA) plate and incubate at 30 ± 2 °C for 7 days. After incubation, examine the recovery membrane for any colonies.

Control experiments:

No titration (determines viability changes due to mechanical shear or other effect not related to acidification).

Operate the system for a cycle, but with a 'mock' titration which does not add acid.

Positive control (determines viability changes due to pH):

1. Perform mixing of *B. diminuta* spiked IgG in beaker for 1 hour.
2. Test for viable *B. diminuta* by performing serial dilutions and also testing undiluted volumes via membrane filtration, followed by plating onto TSA with incubation at 30 ± 2 °C for 7 days.

Negative control (determines viability changes in a standing sample):

1. Perform VI of *B. diminuta* spiked IgG in beaker for 1 hour.
2. Test for viable *B. diminuta*.

Table 3

Results of the B.diminuta experiments for dead-leg testing.

	***B.diminuta* Titer (CFU/mL)**	
VI System	**Input (CFU/mL)**	**Recovery**
Cycle 1	1.2×10^7	0
Cycle 2	1.0×10^7	0
Post cleanliness	N/A	0
No titration control	1.3×10^7	1.9×10^7
Small Volume Controls		
Positive control	1.2×10^7	0
Negative control	1.0×10^7	1.5×10^7

The experiment confirms that the system does not contain hold-ups. No *B.diminuta* was detected after the two VI cycles or the post cleanliness wash out of the system, despite every effort to test the complete manifold including the recirculation loop. The positive control without acidification was unaffected, indicating that mixing, pumping and time do not inactivate *B.diminuta* and this result is confirmation that the system is effective for low pH inactivation and does not contain any hold-ups that can result in the contamination of inactivated elutions with non-inactivated elutions.

Bacteriophage Test

To demonstrate the effectiveness of the system a virus analogue was used. Phi6 was chosen to simulate the inactivation kinetics of enveloped mammalian viruses and allow a test that closely resembles the end-user application, Table 4. Details of the bacteriophage are shown below. Using the bacteriophage method allows a specific log reduction value (LRV) to be determined.

Table 4

Properties of the bacteriophage Phi6.

Bacteriophage	Genome	Approximate Size (nm)	Enveloped	Mammalian Viruses with Shared Properties*
Phi6	RNA	75-85	Yes	HIV & BVDV

Note: *Based on genome and presence or absence of envelope.

The bacteriophage testing was performed in a similar way to the *B. diminuta* testing. Two low pH cycles were performed. The main differences being that the low pH inactivation for Phi6 was performed at pH 3.5 and the wash out of the system was performed with PBS. Samples were analyzed for Phi6 using plaque assays with the host *Pseudomonas syringiae*, followed by overnight incubation at 37 °C. For Phi6 the data are shown in the table below (Table 5).

Table 5

Results of the Phi6 test of system performance

Phi6	Start Titer (PFU/mL)	End Titer (PFU/mL)
Cycle 1	1.3×10^7	0
Cycle 2	2.0×10^7	0
Biocontainer wash post cycle 2	NA	0
Biocontainer control (no titration)	3.4×10^7	3.3×10^7

No Phi6 were detected after low pH virus inactivation, indicating the system is effective for inactivating bacteriophage and that there is no significant contamination of inactivated elution with non-inactivated elution. Two cycles were performed in a single biocontainer indicating that there was no carryover of bacteriophage from cycle to cycle. Additionally a biocontainer wash after the second inactivation cycle showed no remaining active bacteriophage. A negative control where the same pump speeds and mixer speeds were performed, but without any low pH inactivation, was performed to confirm that the phage was not being inactivated over time or due to shear forces from mixing and pumping. This control confirms that the titer is not changed without the low pH step and ratifies that the system is effective for virus inactivation through low pH.

Test of 24 Hour Operation

Here we show operation for over 24 hours and confirm that the system can run robustly without user input for prolonged periods of time. To mimic chromatographic elutions from KANEKA◆ KanCap A Protein A columns, an IgG test solution was created. 0.1x PBS was included as elutions from Protein A columns typically 'front' ahead of the elution buffer and the final wash buffer is often PBS. The elution buffer is typically 50 mM acetic acid and the elution pool is normally around pH 4. Therefore it is reasonable to mimic the elution pool by titrating to pH 4 with acetic acid. To mimic the output of the Cadence BioSMB continuous chromatography system a pump was programmed to supply a semi-continuous feed-stream to the VI system. The pump flow rate was set to 280 mL/min. This was delivered cyclically with 8 minutes pumping followed by 4 minutes of not pumping, for a delivery of 10 L per hour.

Preparatory experiment to determine titration set-points

Before operating the system a test titration was performed in order to develop a titration strategy. 100 mL of mock elution was titrated down to pH 3.5 with 1 M acetic acid and back up to pH 8 with 1 M Tris. It was determined that around 2.5% of the original volume of acetic acid had to be added (2.5 mL) to achieve pH 3.5 and 5.2% of Tris had to be added to achieve pH 8. This led us to the following titration strategy, Table 6. The values are typical of the data set that the end user will need to generate to enter into the system when commissioning for a particular product.

Table 6

Titration strategy with 1 M acetic acid and 1 M Tris base.

pH Adjustment	1M Acetic Acid (% addition W/W)	Set-Point pH	1M Tris Base (% Addition W/W)	Set-Point pH 2
Step 1	1.25		2.6	
Step 2	0.3	3.6	1	7.3
Step 3	0.15	3.5	0.3	8

Transfer of titration strategy to the VI system

The titration is performed in three steps. Step 1, a bolus of titrant is added, this and all the others can be performed as a percent of the weight in the mixer. For rapid pH adjustment it is effective to make half of the expected addition in this first step. Step 2, a smaller amount of titrant is added and the pH checked after a period of mixing. Additional rounds of addition and mixing are performed until the Step 2 set-point is reached. For the acidification, the Step 2 set-point can be set relatively closely to the final desired pH as acetic acid is a relatively weak acid and the pH of the solution is becoming close to that acid itself. For titrating back up Tris is a much more effective buffer at pH 8 and so more care has to be taking in adjusting the pH back up as there is a greater risk of overshoot. Step 3, smaller amounts of titrant are added, mixed and the pH checked. Further additions are made until the final step 3 checkpoint is reached. To avoid overshooting the pH set-point, normally these additions are relatively small.

To begin the test a new manifold was installed. After installation a VI sequence was initiated. This opens a flow path into the system and sets the VI to wait for the elutions or product pool to arrive in the mixer. The pH probes were allowed to sit in the elutions, fully immersed for about 10 minutes before a single set-point correction was performed to calibrate the system. The 4 minutes between elutions supplied suitable time to make this correction prior to the first titration. A sample was taken from the sampling port. This was performed 30 seconds after the end of the elution to make sure the system was homogenous. A time stamp was created on the pH transmitter immediately after removing a sample of product from the system. The pH of the sample was then measured offline and the offset on the transmitter against the pH that was recorded by the system at the time stamp. This way if the pH in the system changes between sampling and making the pH offset, the pH offset is still appropriate. The methodology means that the pH probes can undergo a single point calibration without pausing the process.

With the offset made, the system did not have to be calibrated during the rest of the 24 hour operation.

Samples were taken periodically to ensure that the pH of the system probes was accurate and represented the actual pH of samples in the mixers, Table 7. The data show that pH probes stay within 0.05 pH units of the reading from the externally calibrated probe. This is within the desired accuracy of +/-0.1 pH units.

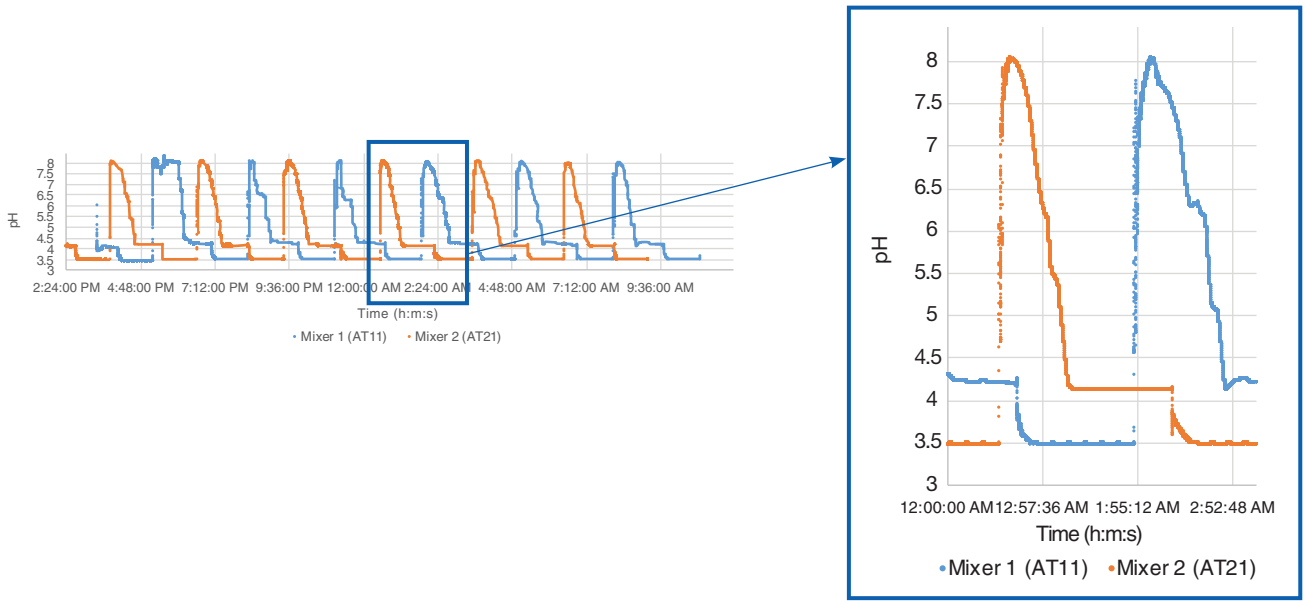
Table 7

Data comparing pH data from the internal system probes and external probe measurement of samples.

Mixer 1 Time (h:m)	Internal Probe pH	External pH	Mixer 2 Time (h:m)	Internal Probe pH 2	External pH 3
19:16	3.50	3.48	17:20	3.45	3.43
0:36	3.50	3.48	21:09	3.48	3.47
3.32	3.52	3.48	23:12	3.47	3.47
6:35	3.48	3.48	2:15	3.48	3.45
10:25	3.51	3.47	4:59	3.47	3.46
12:10	3.43	3.48	8:58	3.48	3.46
15:30	3.43	3.48	16:10	3.47	3.42
Average	3.481	3.479		3.471	3.452
Std Dev (SD)	0.034	0.003	0.313	0.010	0.019
Bias	0.003			0.020	
SD	0.034			0.010	
Total Error (Bias + 2xSD)	**0.071**			**0.039**	

Figure 8

Data showing the operation the Cadence virus inactivation system via the two in-built pH probes.



The data from the two pH probes, one in each mixer, is shown above, Figure 8. The pH of the incoming material is pH 4. The material is titrated down to pH 3.5, held at this pH for 60 minutes and then titrated up to pH 8. After pH 8 is achieved the mixer is emptied and the probe is in air for a period of time, until new elutions cover the probe again and the cycle starts over. The pH traces appear as expected and no pH overshoots or process deviations are observed throughout the process. During the titration adjustments product was pumped through the recirculation loop and the mixer was operated to ensure the product was homogenous. The titration time is a function of the strategy and this is a balance between hitting the set-point accurately and titrating quickly. Here the focus was on titrating quickly and most of the titrations were completed in under 5 minutes. All of the titrations resulted in a product at a pH within 0.07 pH units of the set-point. Samples were taken periodically and the pH of the product during the hold as measured by the internal probe was compared to an external calibrated pH meter. Here we find that the internal probe always read within 0.5 pH units of the external probe indicating that the internal probe is accurate and does not drift through the course of a 24 hour experiment.

After titration down the system can be programmed to wait for a certain period of time to check that the pH has not drifted before performing the low pH hold, or emptying the tank. The check time was set for one minute and this proved a useful method for ensuring the pH was at the set-point: After the one minute check time the pH did not drift. Additionally, investigations of the manifold after the test showed no apparent damage to the manifold and no leaks were observed during the 24 hours of operation.

Conclusion

The Cadence virus inactivation system is a robust solution for the operation of fully automated semi-continuous low pH virus inactivation. The system has a single-use flow path which contains no hold-ups. The overall strategy for low pH hold was chosen to mimic a current batch process, where whole elutions are pooled before being processed via low pH. This strategy is proven to be robust and reliable and here it is made applicable to continuous processing through the use of an alternating mixer strategy coupled with design elements to minimize carry over and foaming risks.

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